Journal of Food Protection, Vol. 72, No. 5, 2009, Pages 1012–1019

Thermal, Chemical, and Photocatalytic Inactivation of *Lactobacillus plantarum* Bacteriophages

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MS 08-537: Received 23 October 2008/Accepted 11 January 2009

ABSTRACT

The effect of several biocides, thermal treatments, and photocatalysis on the viability of four *Lactobacillus plantarum* phages was investigated. Times to achieve 99% inactivation (T_{99}) of phages at 63, 72, and 90°C were evaluated in four suspension media: deMan Rogosa Sharpe broth, reconstituted skim milk, a commercial EM-glucose medium, and Tris magnesium gelatin buffer. The four phages studied were highly resistant to 63°C ($T_{99} > 45$ min); however, counts < 10 PFU/ml were achieved by heating at 90°C for 5 min. Higher thermal resistance at 72°C was observed when reconstituted skim milk and EM-glucose medium were assayed. Peracetic acid (0.15%, vol/vol) was an effective biocide for the complete inactivation of all phages studied within 5 min of exposure. Sodium hypochlorite (800 ppm) inactivated the phages completely within 30 min. Ethanol (100%) did not destroy phage particles even after 45 min. Isopropanol did not have any effect on phage viability. Phage counts < 50 PFU/ml were obtained within 180 min of photocatalytic treatment. The results obtained in this work are important for establishing adequate methods for inactivating phages in industrial plants and laboratory environments.

Lactobacillus plantarum is a flexible and versatile lactic acid bacterium that is found in a variety of environmental niches including dairy, meat, and many vegetable fermentations. The ecological flexibility of *L. plantarum* is a reflection of one of the largest genomes known among lactic acid bacteria (LAB) *(23). L. plantarum* can survive gastric transit and can colonize the intestinal tract of humans and other mammals *(13).* Several researchers have described probiotic properties of *L. plantarum (9, 16, 19, 33, 34, 41, 50),* including the effect of its consumption on human physiology *(13).*

L. plantarum as an adjunct culture in cheese manufacture may enhance cheese quality *(12, 32, 35).* Milesi et al. *(35)* reported that in Cheddar and Cremoso Argentino cheeses, *L. plantarum* survived and enhanced secondary proteolysis. Fiore Sardo and mozzarella cheeses have been manufactured with multistrain cultures containing *L. plantarum.* In Fiore Sardo cheese, balanced lipolytic, proteolytic, and fermentation activities conferred desired attributes, and fewer spoilage microorganisms were found *(32).* Improved texture and sensory properties and extended shelf life were obtained in mozzarella cheese *(12).* Thus, *L. plantarum* could be used as a probiotic starter culture in the production of functional foods, with advantages over the majority of the probiotics currently in commercial use. However, the activity of *L. plantarum* could be severely affected by phage infections.

Bacteriophage infection of LAB has been a major problem in the dairy industry, causing slow fermentation or complete starter failure and thus economic losses *(26, 36).* Because of the constant risk of economic losses, control of phages is a major area of concern in handling LAB *(7).*

Bacteriophages that infect *L. plantarum* have been isolated from various sources: meat *(10, 49),* silage *(8, 10, 14),* homemade cheese whey *(8),* fermented vegetables *(27, 52),* and fermented maize and coffee *(10).* These phages have been characterized mainly by their genomes *(8, 10, 14, 26, 27, 39, 49, 52)* and by their interactions with sensitive bacterial strains *(8, 27, 39, 49)*; however, data on their chemical and thermal resistance are scarce. In contrast, data have been reported on thermal and chemical resistance of phages infecting *Lactococcus (2, 5, 11, 15, 20, 43, 47, 51, 53), Streptococcus thermophilus (3), Lactobacillus casei* and *Lactobacillus paracasei (7), Lactobacillus delbrueckii (44),* and *Lactobacillus helveticus (45).*

Bacteriophages remain in the air for long periods, and aerosolization is one of the main dispersal mechanisms of phage particles *(37, 38).* Photocatalysis is commonly used for the destruction of airborne fungi, bacteria, and spores *(17, 25).* However, the efficiency of this treatment for the inactivation of viruses in bioaerosols has not been well studied. This information would be useful for the control of bacteriophages present in the air of dairy environments.

The aim of this work was to study the resistance of *L. plantarum* bacteriophages to thermal and chemical treatments usually applied in dairy plants and laboratories and to analyze the behavior of these phages during photocatalytic treatments.

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MATERIALS AND METHODS

Bacteria strains, bacteriophages, and culture conditions. *L. plantarum* ATCC 8014 was used as the host strain for the collection phages ATCC 8014-B1 and ATCC 8014-B2 (herein referred to as B1 and B2, respectively) and two phages (FAGK1 and FAGK2) isolated from kefir grains. *L. plantarum* was grown and routinely reactivated overnight (37°C) in deMan Rogosa Sharpe (MRS) broth (Britania S.A., Buenos Aires, Argentina) and maintained as frozen stock (−80°C) in MRS broth plus 15% (vol/vol) glycerol. MRS broth and MRS agar supplemented with 10 mM $CaCl_2$ (MRS-Ca) were used to replicate and count phage particles, respectively. Phage stocks were prepared as described by Neviani et al. *(40)* and stored at 4°C (MRS broth) and −80°C (MRS broth plus 15% glycerol). Phage counts, expressed as PFU per milliliter, were obtained with the double-layer plaque titration method *(48).* Incubations were conducted at 37°C.

Thermal treatments. To study the thermal resistance of phages, three temperatures (63, 72, and 90°C) were used with four suspension media: (i) MRS broth, (ii) reconstituted (10%, wt/vol) dry skim milk (RSM), (iii) reconstituted (6%, wt/vol) commercial complex EM medium plus 1% (wt/vol) glucose (EM-glucose), and (iv) Tris magnesium gelatin (TMG; 10 mM Tris-HCl, 10 mM $MgSO_4$, and 0.1% [wt/vol] gelatin, pH 7.4) *(6).* The temperatures were selected based on the heat treatment conditions used in the dairy industry. The suspension media studied are usually used in the laboratory or dairy industry.

Each phage (approximately 1×10^7 PFU/ml) was mixed with the suspension medium, and 1-ml volumes of the mixture were distributed in Eppendorf tubes and incubated at one of the three temperatures. At predetermined intervals, the tubes were removed and cooled in ice water. The surviving bacteriophages were immediately counted by the double-layer agar plate method *(48).* The results were expressed as the concentration of active viral particles and plotted against time. Times (minutes) to achieve 99% inactivation (T_{99}) of phages were calculated graphically from survival curves as described by Capra et al. *(7).*

Chemical treatments. The biocides used were ethanol (10, 50, 75, and 100%, vol/vol; Cicarelli, Buenos Aires, Argentina), isopropanol (10, 50, and 100%, vol/vol; Cicarelli), commercial sodium hypochlorite (200 to 800 ppm of residual free chlorine), and peracetic acid (0.15%, vol/vol; Proxitane 1512, Química General, Santa Fe, Argentina). Sodium hypochlorite was diluted in phosphate buffer (pH 7). Alcohols and peracetic acid were diluted in distilled water. The resulting pH of the peracetic acid solution was 2.7. All assays were carried out at 25°C, but peracetic acid was assayed also at 40°C according to the suggestion of Schröder *(46).*

After being mixed with the biocide solution, each phage (approximately 1×10^7 PFU/ml) was distributed into Eppendorf tubes (1 ml final volume) and incubated at the selected temperatures for each chemical agent. Phage suspensions in dilution medium without the biocides but after a previous pH adjustment were used as controls to test the effect of pH on phage viability. At predetermined intervals, tubes were removed and the surviving bacteriophages were immediately diluted and counted. The results were expressed as concentration of infectious bacteriophages and plotted against time. The T_{99} of the phages was calculated from the survival curves.

Photochemical and photocatalytic inactivation. The laboratory reactor consisted of an emission source (seven UV lamps of 8 W with maximum emission at 350 nm) and an irradiation chamber, which is a metallic box closed at the top to maintain internal humidity. Phage suspensions diluted in distilled water (1×10^7 PFU/ml) were taken in a slide glass plate with or without catalytic TiO_2 film and covered with a borosilicate glass to create a thickness similar to the size of bioaerosol drops. Samples were then placed in the irradiation chamber and irradiated with UV light for 3 h. Temperature and relative humidity inside the irradiation chamber were controlled and maintained at 40°C and 89.5%, respectively, during all experiments. At predetermined intervals, the concentration of viable bacteriophages was determined and plotted against time.

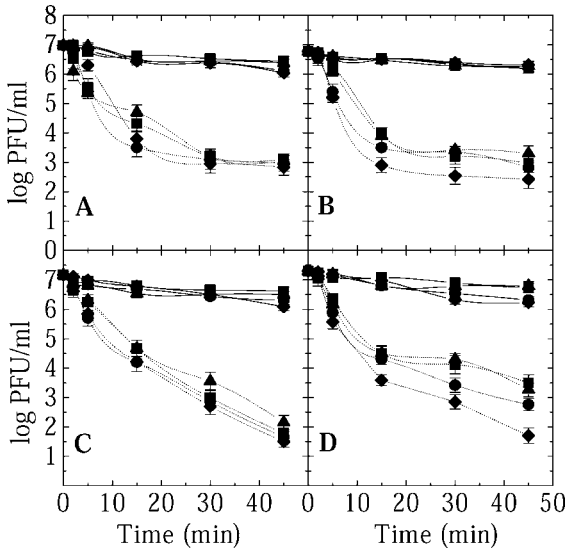

FIGURE 1. *Thermal inactivation kinetics of phages B1 (A), B2 (B), FAGK1 (C), and FAGK2 (D) at 63°C (——) and 72°C (– – –) in MRS (deMan Rogosa Sharpe) broth (♦), RSM (reconstituted dry skim milk) (■), EM-glucose medium (reconstituted commercial complex medium with added glucose) (▲), and TMG (Tris magnesium gelatin) buffer (●). The values are the mean of three determinations.*

Statistical analysis. All data were analyzed using the Statgraphics Plus software (version 3.0, Statistical Graphics Corp., Warrenton, VA). Experiments were replicated three times. Means were compared using a one-way analysis of variance followed by Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

RESULTS

Thermal treatments. Figure 1 shows the viability losses when phage suspensions were heated at 63 and 72°C in MRS broth, RSM, EM-glucose medium, and TMG buffer. A strong resistance at 63°C was exhibited by all the phages ($T_{99} > 45$ min) in the four suspension media used (Table 1). When phage suspensions were heated at 72°C, significant reductions in the number of viable phage particles were obtained but were not sufficient to achieve complete inactivation within 45 min (4- to 5.5-log viability losses). T_{99} values of 5.6 to 11.7 min were obtained at 72°C depending on the phage and suspension medium. Phages B1 and FAGK2 reacted differently to the four suspension media assayed ($P < 0.05$), but no differences were observed between MRS broth and TMG buffer ($P > 0.05$) for phages B2 and FAGK1. In those suspension media, low heat resistance was observed for all phages, with the ex-

TABLE 1. *Thermal resistance of* Lactobacillus plantarum *phages in different suspension media*

Phage	T_{99} (min)[a]									
	MRS broth			RSM		EM-glucose		TMG buffer		
	63°C	72°C	90°C	63°C	90°C	63°C	72°C	90°C	63°C	72°C	90°C
B1	>45	10.0 ± 0.6 a A	<5	>45	<5	>45	8.6 ± 0.6 a C	<5	>45	5.8 ± 0.2 a D	<5
B2	>45	6.0 ± 0.4 b A	<5	>45	<5	>45	10.5 ± 0.3 b C	<5	>45	6.5 ± 0.3 b A	<5
FAGK1	>45	7.5 ± 0.4 c A	<5	>45	<5	>45	11.7 ± 0.5 c B	<5	>45	6.9 ± 0.4 b A	<5
FAGK2	>45	5.6 ± 0.3 b A	<5	>45	<5	>45	8.2 ± 0.4 a C	<5	>45	6.6 ± 0.4 b D	<5

[a] Time to achieve 99% inactivation of phage particles. The values are the mean ± standard deviation of three determinations. Within a column, means with different lowercase letters are significantly different ($P < 0.05$). Within a row, means with different uppercase letters are significantly different ($P < 0.05$). MRS, deMan Rogosa Sharpe; RSM, reconstituted dry skim milk; EM-glucose, reconstituted commercial complex medium with added glucose; TMG, Tris magnesium gelatin.

FIGURE 2. *Inactivation kinetics of phages B1 (A), B2 (B), FAGK1 (C), and FAGK2 (D) with 10% (◆), 50% (■), 75% (▲), and 100% (●) (vol/vol) ethanol. The values are the mean of three determinations.*

ception of phage B1, which had the highest thermal resistance in MRS broth. Treatment at 90°C for 5 min was enough to obtain phage counts < 10 PFU/ml in all the suspension media tested for all the phages assayed (Table 1).

Chemical treatments. Among the ethanol concentrations assayed in this work, 100% ethanol produced the highest phage inactivation (Fig. 2), although it did not achieve a complete loss of viability within 45 min (3- to 4-log reductions). Treatment with 100% ethanol resulted in T_{99} values of 3.7 to 7.4 min (Table 2). No effects on phage viability ($T_{99} > 45$ min) were observed when 10% ethanol was used. However, 50% ethanol ($T_{99} = 8.3$ to 14.8 min) was more effective than 75% ethanol ($T_{99} = 29.0$ and 44.6 min) with the exception of phage FAGK2 (75% ethanol, $T_{99} > 45$ min).

All phages were barely affected by isopropanol. For all concentrations assayed, T_{99} values were >45 min (data not shown).

For sodium hypochlorite, 200 ppm had a considerable effect on phage viability (2-log reductions and $T_{99} > 45$ min). When 400 ppm was assayed, 3-log reductions (phages B2, FAGK1, and FAGK2) and 4-log reductions (phage B1) and T_{99} values of 2.5 to 4.3 min were obtained (Table 2). At 800 ppm, sodium hypochlorite produced undetectable counts (<10 PFU/ml) within 15 min (phage B2) and 30 min (phages B1, FAGK1, and FAGK2) (Fig. 3).

Peracetic acid (0.15%) was the most effective biocide agent tested, and caused the fastest phage inactivation. None of the phages were detectable within 5 min of treatment, both at 25 and 40°C. T_{99} values were <5 min for both temperature conditions (data not shown).

Photochemical and photocatalytic inactivation. Regarding the photochemical treatment, 2.3- to 3-log viability losses were observed after 3 h of exposure. When TiO_2 was

TABLE 2. *Resistance of* Lactobacillus plantarum *phages to ethanol and sodium hypochlorite*

	T_{99} (min)[a]						
	Ethanol				Sodium hypochlorite		
Phage	10%	50%	75%	100%	200 ppm	400 ppm	800 ppm
B1	>45	8.3 ± 0.2 a A	29.0 ± 0.5 a B	3.7 ± 0.2 a C	>45	2.5 ± 0.2 a X	1.8 ± 0.1 ab Y
B2	>45	14.8 ± 0.3 b A	44.6 ± 0.4 b B	7.4 ± 0.3 b C	>45	4.3 ± 0.2 b X	1.8 ± 0.1 ab Y
FAGK1	>45	11.4 ± 0.5 c A	41.6 ± 0.9 c B	5.0 ± 0.3 c C	>45	2.6 ± 0.3 a X	1.9 ± 0.1 a Y
FAGK2	>45	14.7 ± 0.7 b A	>45	7.3 ± 0.2 b B	>45	4.2 ± 0.5 b X	1.6 ± 0.2 b Y

[a] Time to achieve 99% inactivation of phage particles. Values are the mean ± standard deviation of three determinations. Within a column, means with different lowercase letters are significantly different ($P < 0.05$). Within a row and a biocide, means with different uppercase letters (A, B, or C; X or Y) are significantly different ($P < 0.05$).

added, undetectable counts (<50 PFU/ml) of viral particles were reached after 60 min (phages B1 and B2) and 180 min (phages FAGK1 and FAGK2) (Fig. 4).

DISCUSSION

Lactobacilli are widely used in a variety of food fermentation processes and contribute to the flavor and texture of products. The organic acids produced and the resulting low pH serve to protect fermented products from spoilage microorganisms *(52).* The industrial importance of lactobacilli is enhanced by their use as probiotics, and a variety of *L. plantarum* strains are marketed as probiotics *(13). L. plantarum* also is currently used as starter in a variety of food fermentations, specifically improving cheese quality when used as an adjunct culture *(12, 32, 35).* However, the increasing use of *L. plantarum* as a starter or adjunct culture can lead to bacteriophage infections in cheesemaking settings, with adverse effects on the final product. Effective control measures are necessary to minimize the problems deriving from phage attacks. To establish such measures, thermal and chemical resistance and the efficiency of new methodologies for the inactivation of phage particles must be investigated.

The thermal resistance of four *L. plantarum* phages in diverse suspension media was studied. A comparison of the behavior of these phages with that of other mesophilic LAB phages revealed that *L. plantarum* phages were highly resistant at 63 and 72°C. Capra et al. *(7)* reported low heat resistance for *L. casei* and *L. paracasei* phages; they were inactivated after 5 min of heating at 72°C in MRS broth and RSM and after 15 min in TMG buffer. Lu et al. *(27)* reported that *L. plantarum* phage ϕJL-1, isolated from cucumber fermentation, was rapidly inactivated at temperatures above 70°C. The *D*-values were 2.7 min at 70°C and 0.2 min at 80°C. Phage titers decreased below the detection limit (20 PFU/ml) after heating for longer than 60 s at 80°C or longer than 15 s at 90 or 100°C. Lower thermal resistance was observed for phage fri, which was isolated from a meat starter culture *(49).* This phage was very sensitive

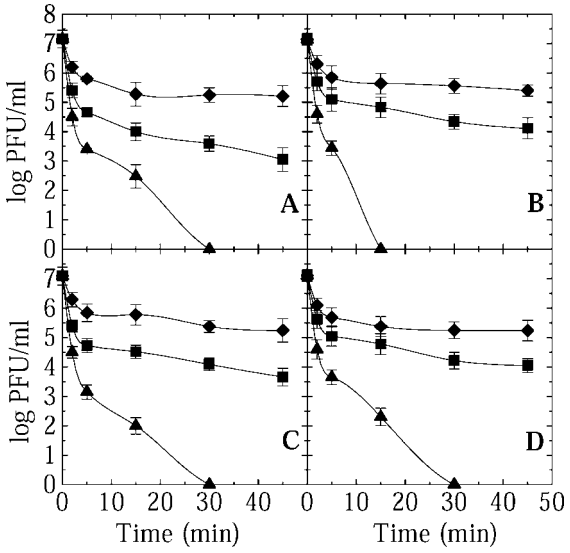

FIGURE 3. *Inactivation kinetics of phages B1 (A), B2 (B), FAGK1 (C), and FAGK2 (D) with 200 ppm (◆), 400 ppm (■) and 800 ppm (▲) of residual free chlorine. The values are the mean of three determinations.*

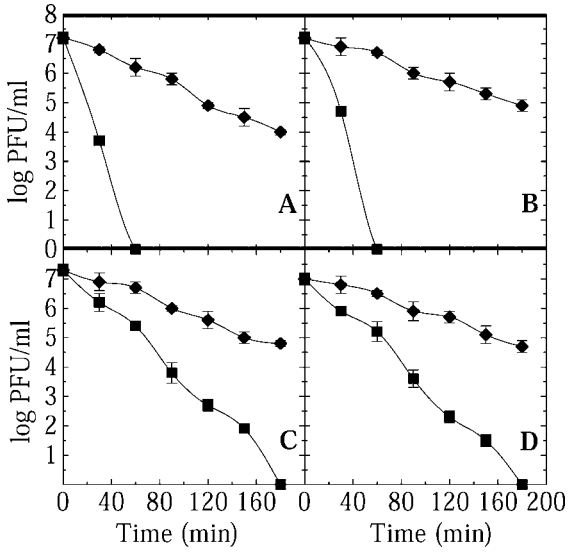

FIGURE 4. *Photochemical (UV) (▲) and photocatalytic (UV-TiO_2) (■) inactivation kinetics of phages B1 (A), B2 (B), FAGK1 (C), and FAGK2 (D). Phages diluted in distilled water (1×10^7 PFU/ml) were taken in a slide glass plate with or without catalytic TiO_2 film simulating bioaerosol drops and irradiated with UV radiation (maximum emission of 350 nm). At intervals, the numbers of surviving phages were determined. The values are the mean of three determinations.*

to 60°C, with a survival rate of only 30% after 5 min and near elimination of the population after 10 min. At 70°C, the phage particles were inactivated within 5 min.

As previously reported *(3, 7, 44, 45, 47)* and according to our results on *L. plantarum* phages, neither low-temperature, long-time treatment (63°C for 30 min) nor high-temperature, short-time treatment (75°C for 15 s) applied in cheesemaking are sufficient to completely inactivate phage suspensions. Consequently, a high concentration of phage particles could remain in milk, leading to the failure of fermentation and/or the absence of specific beneficial properties in the final product *(7).* Except for phage Ib_3 (*L. delbrueckii*), a heat treatment at 90°C for 5 min inactivated high-titer suspensions of all phages studied in all tested media *(3, 7, 44, 45, 47).* However, that treatment can be used in fermented milk manufacture but not for milk used for cheesemaking because of the denaturation of whey proteins, which hampers the manufacturing process. Similar results were reported for lactococcal phages by Buzrul et al. *(5),* who found that only 2 of the 10 phages studied were totally inactivated (<10 PFU/ml) at 72°C for 15 min in M17 broth, whereas 90°C for 5 min inactivated half of the phages in the same suspension medium. Atamer et al. *(1)* studied the thermal resistant of 56 *Lactococcus lactis* phages. These researchers found that about 40% of the phages were resistant to treatment at 90°C for 5 min. P1532 and P 680 phages were detectable even after heating for 5 min at 97 and 95°C, respectively. Consequently, and in agreement with Atamer et al. *(1),* it seems that the standard protocol *(48)* for phage detection in dairy samples would not always be appropriate for distinguishing highly thermoresistant phages from nonphage inhibiting agents because the protocol includes a heat treatment of the sample at 90°C for 15 min to inactivate phages.

In the present study, high thermal resistance in RSM and EM-glucose media was observed for all phages except phage B1. Consequently, the suspension media used in the industry could have a protective effect on *L. plantarum* phages. The same result for phage suspensions in milk was reported for streptococcal *(11)* and *Lactococcus lactis (15, 47)* phages. Fabrizio et al. *(15)* reported a moderate increase in the heat resistance of *Lactococcus lactis* phages when whole milk was used instead of skim milk. For Argentinian *Lactococcus lactis* phages *(47),* M17 broth was generally less effective for protecting phages from heat than was TMG buffer. In contrast, for phages of *L. helveticus (45), S. thermophilus (3),* and *L. casei* and *L. paracasei (7)* no significant influence of the suspension medium was found.

In the investigation of various chemical agents commonly used as biocides in industries and laboratories, peracetic acid was the most effective biocide; it completely inactivated phage suspensions of high titers in a short time. Identical behavior was at 25 and 40°C. Consequently, we suggest the use of this biocide at 25°C because of its ease of use in industrial environments. However, Schröder *(46)* proposed 40°C as the most effective temperature for inactivation of bacteria, yeasts, and fungi. This biocide was similarly effective on other LAB phages *(3, 7, 44, 45, 47).* Maillard et al. *(28)* found that peracetic acid was highly effective against phage F116 (*Pseudomonas aeruginosa*); a concentration of 0.1% achieved a 99.99% reduction in phage titer after 10 min of contact. Peracetic acid produced a complete cleavage of the phage nucleic acid. However, it is not clear whether the biocide inactivates the phage DNA inside the capsid or after the DNA is released from a fractured protein coat. Biocide treatment of phage F116 often resulted in a structural alteration of the phage capsid, which was observed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis *(29, 30).*

With regard to alcohol treatments, ethanol was not very effective for inactivating phages; even after 45 min of contact, large numbers of viable particles were counted for the four phages. Concentrations of 50, 75, and 100% ethanol reduced phage suspensions titers only partially, and 10% ethanol had little effect on viral particles. Similar results were reported by Capra et al. *(7)* for *L. casei* and *L. paracasei* phages, whereas *S. thermophilus (3), L. delbrueckii (44), L. helveticus (45),* and *Lactococcus lactis (47)* bacteriophages were more sensitive to ethanol treatment. Ethanol may be used in sanitation of air flow units or surfaces in laboratories, but to be more effective, the cleaning must be used in conjunction with another biocide.

For the *L. plantarum* phages studied in this work, 50% ethanol was more effective than 75% ethanol. These results are astonishing because similar results for other phages had not been previously reported. For *L. helveticus* phages *(45),* 75% ethanol was more effective than 100% ethanol. In agreement with previous results, Buzrul et al. *(5)* reported that treatment with 50 and 75% ethanol increased the loss of viability of *Lactococcus lactis* phages in comparison with treatment with 100% ethanol. However, for *L. casei, L. paracasei,* and *L. delbrueckii* bacteriophages, ethanol at 75 and 100% was more effective than 50% ethanol *(7, 44).*

Isopropanol was not an effective biocide against the phages studied in this work; all of them were resistant to this alcohol. The same result was found previously for *L. casei, L. paracasei,* and *L. delbrueckii* phages *(7, 44).* Low resistance to isopropanol was observed for *S. thermophilus* and *L. helveticus* phages. However, this biocide was less efficient than ethanol *(3, 45).*

In comparison with results obtained in our previous studies *(3, 45, 47),* the four *L. plantarum* phages were more resistant against residual free chlorine than were *S. thermophilus, L. helveticus,* and *Lactococcus lactis* phages. Exposures of 15 min for phage B2 and 30 min for phages B1, FAGK1, and FAGK2 to 800 ppm of residual free chlorine were necessary to completely inactivate phage suspensions; however, this chlorine concentration was higher than that allowed in the food industry. Consequently, the use of this biocide at concentrations assuring phage inactivation in an industrial environment is not possible, although these concentrations may be used in the laboratory to complement ethanol cleaning. Similar results were reported by Capra et al. *(7)*; 5 min of exposure to 800 ppm of residual free chlorine was necessary to totally inactive suspensions of *L. casei* and *L. paracasei* phages. In contrast, sodium hypochlorite at 100 to 300 ppm resulted in undetectable counts of *S. thermophilus (3), L. helveticus (45),* and *Lactococcus*

lactis phages *(47).* In contrast, phage Ib_3 (*L. delbrueckii*) exhibited an extremely high resistance to chlorine *(44),* requiring 45 min at 1,200 ppm to inactivate phage suspensions. Maillard et al. *(31)* found that this biocide caused aggregation of the tail proteins of phage F116. In addition, sodium hypochlorite caused a high number of structural alterations to the bacteriophage head, possibly releasing the nucleic acid of the phage into the surrounding medium.

Photocatalytic sterilization with TiO_2 has attracted attention as an alternative to procedures such as heat treatment, UV radiation, and use of chemical disinfectants. The semiconductor TiO_2 acquires a reducing or oxidizing potential when photoexcited by energy wavelengths below 385 nm, thus catalyzing various chemical reactions including the decomposition of organic compounds and inactivation of organisms *(18).*

The data available on the efficiency of photocatalysis in phage inactivation are very scarce. With regard to LAB phages, Kakita et al. *(21)* studied the inactivation of phage PL-1 (*L. casei*) using black-light radiation (300 to 400 nm) in the presence of TiO_2 thin film. In contrast to our study, Kakita et al. carried out the experiment in a liquid medium. After inactivation assays, the authors observed that the phages were turned into ghost particles; only the empty heads (without genomic DNA) remained. Kashige et al. *(22),* by extending the study of Kakita et al., suggested that phage PL-1 inactivation was caused primarily by damage to the capsid protein followed by injury to the genome DNA. This action was caused by active oxygen species generated in the TiO_2 film in the aqueous medium under the black light. The generation of both O_2^- and $^{\dagger}OH$ in the aqueous medium was confirmed by their chemical analysis. Electrophoresis revealed that the genomic DNA inside the phages particles was considerably fragmented by this treatment.

The only reported study involving photocatalysis (UV radiation and TiO_2) and viruses in bioaerosols is that of Bonazza *(4).* In that work, the multiple nuclear polyhedrosis virus of *Anticarsia gemmatalis* (AgMNPV) was irradiated in the presence or absence of TiO_2 film for 2 h. Photochemical treatment of AgMNPV revealed a 2-log viability loss, whereas the virus was undetectable (>4-log viability loss) when TiO_2 was added. Bonazza also reported first-order kinetics for chemical and photocatalytic inactivation.

The use of photocatalysis has several advantages, such as absence of residues and the possible treatment of mixtures of diverse pollutants due to scarce selectivity and easy operation. The low cost, abundance, and nontoxic properties of TiO_2 make it the compound most frequently used as a catalyst *(4).*

Various types of UVC (254 nm) and UVC-TiO_2 irradiation equipment are available *(24, 42)* for the purification of environmental air. However, in our study UVA radiation (315 to 400 nm) was assayed. Unlike of UVC radiation, UVA radiation is safe to use in the presence of personnel, which would allow the operation of lamps for long periods. Therefore, the TiO_2 photocatalysis conditions used in our study may be feasible for the inactivation of *L. plantarum* phages. However, further research is necessary to adapt this equipment for use as a new methodology for phage inactivation in industrial environments.

The data obtained in this work provide information useful for designing more effective treatments to be applied in laboratories and industrial plants that handle *L. plantarum.* Thermal treatment at 90°C for 5 min should assure the inactivation of *L. plantarum* phages. Thus, this treatment may be applied to raw materials used in fermented milk manufacture and to the growth medium used in *L. plantarum* starter culture production. However, this treatment is not suitable for milk used in cheesemaking because of protein denaturation. Treatments at 63 and 72°C will not assure phage inactivation. Peracetic acid is an effective biocide that may be used for cleaning industrial environments and equipment. This biocide has the advantages of efficiency at low concentration and decomposition into nontoxic residues. In contrast, the application of sodium hypochlorite would not be suitable in plant environments because the concentrations necessary to assure phage inactivation are higher than those allowed in the food industry; however, it could be helpful in the laboratory, as would ethanol. TiO_2 photocatalysis may be a new and efficient alternative for phage inactivation in industrial and laboratory environments.

ACKNOWLEDGMENTS

This work was supported by the Universidad Nacional del Litoral (Santa Fe, Argentina; Programación CAI+D 2006, Secretaría de Ciencia y Técnica, Project 37-200), the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (Project PIP 2004, 5321), and the Agencia Nacional de Promoción Científica y Técnológica of Argentina (Project PICT 2004, 20358). We thank Dr. Orlando Alfano, Dr. Horacio Irazoqui, and Dr. Gustavo Imoberdorf for specialized assistance in the photocatalytic experiments.

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